Identification of the fungal population in air samples collected at Pediatric Dentistry teaching clinic

Lariane Marcolino Nunes*; Talita Silva Viana; Gisselly Maria Campos da Silva***; Maria Paula Jacobucci Botelho****; Lígia Maria Molinari Capel*****

* Graduated in Dentistry, Centro Universitário de Maringá
** Graduated in Biological Sciences, Centro Universitário de Maringá
*** Dentistry student, Centro Universitário de Maringá
**** Professor, Centro Universitário de Maringá
***** Adjunct Professor, Centro Universitário de Maringá

Received November 18, 2017. Approved March 28, 2018.

ABSTRACT

Infectious agents can be transmitted via microscopic particles suspended in the air and secretions present on contaminated surfaces of instruments and equipment. Indoor airborne contaminants include fungi, bacteria and viruses, which come from outside air, the air conditioning system, the building, furniture, carpet and their occupants. Most procedures performed at the dental clinic release large amounts of aerosols, minute particles that remain for several hours in the air and may contain several microorganisms, among them fungi. In teaching clinics, this contamination is greater due to the high number of occupants and procedures performed at the same time. By knowing the fungal genera to which their patients are exposed, the professional can minimize the risks of infection, adopting practices of hygiene of the environment and air that can aid in the prevention of diseases. The fungi check was performed by the plate sedimentation method, which is useful for the analysis of the quantity and quality of fungi present in internal and external environments. Species belonging to the genus Aspergillus, Fusarium, Cladosporium, Nigrospora and Penicillium were identified, most frequently found Penicillium and Aspergillus genera from external and internal environment.

Descriptors: Bacterial Infections and Mycoses. Aerosols. Dental Clinics. Pediatric Dentistry. Infection Control.

1 INTRODUCTION

Aerosols are defined as liquid or solid particles suspended in the air, produced by humans, animals, instruments or machines. Humans generate bioaerosols while speaking, breathing, sneezing or coughing. Due to the nature of their profession, health professionals are more exposed to pathogenic microorganisms. The risk of exposure is in line with the infectious nature of their patients, interventions or instruments that produce bioaerosols[1].

Higher Education Institutions (HEIs) have to train professionals from different areas with adequate scientific knowledge to develop their work activities. Thus, biosafety-related maneuvers for patients, students and professionals should be incorporated into routine dental practice since the undergraduate period[2]. Certain professionals, because of their profession, need to spend much of their time indoors such as dental clinics, hospitals and offices, which are often artificially heated. Poor air quality can cause various respiratory infections[3]. Thus, in closed environments where there is no air circulation and renewal, the chances of contamination are higher, increasing the propensity for various diseases. Among them, fungal infections can present severe and fatal complications in immunocompromised patients, with great impact on morbidity and mortality[4].

Fungi are able to grow in indoor environments where there is sufficient humidity and a nutrition source, such as wood, paint and acoustic and thermal insulation materials, and the release of spores occurs as part of their reproductive process[5].

Dental professionals should work with three-layer face masks that protect against droplet contamination but do not provide protection against aerosols[6]. Thus, there is concern in reducing the contamination of the dental environment, aiming at the protection of the team and patients.

Fungal spore detection by the plate sedimentation method has proved useful for the analysis of the quantity and quality of fungi present in indoor and outdoor environments. By means of this type of research, it is possible to compare the data obtained with border counts set by the Ministry of Health, which brings as a maximum acceptable value for biological contamination 750 CFU/m^3 of fungi. If this value is exceeded, the environment is considered unfit for health[7].

Indoor air pollution stems from the combination of physical, chemical and biological effects, as well as other factors such as inadequate ventilation of the environment. The main sources of external air pollution are traffic, industrial and construction activities[8]. Fungi are usually transported in a building by heating, air conditioning and ventilation systems, windows, doors and contaminants in building materials[5]. Preventive measures should therefore be taken in places prone to contamination of the internal environment, which may be harmful to human health.

Considering that several characteristics of dentistry teaching clinics influence air quality, such as quantity and flow of people (patients, students, teachers and other employees); in addition to environmental conditions such as air circulation and relative humidity, frequency and quality of cleaning, care with the microbiological quality of the air must be doubled.

In this way, the analysis of the quantity and quality of fungi present in a given indoor environment can inform the necessity or not

of improving the cleaning and disinfection process and, thus, guarantee the health of the patients and the professionals. This study aimed to identify and quantify the prevalent fungal genera in the air in a Pediatric Dentistry teaching clinic of a HEI.

2 MATERIAL AND METHODS

The air samples were taken in a pediatric dentistry clinic of an undergraduate course in Dentistry, in the city of Maringá, with samples in duplicate during a semester, always in the afternoon, due to the greater flow of patients in this period.

For the collection of air fungi, the plate sedimentation method was used by exposing the culture media specific for each analysis in the environments, isolating the microorganisms of the air[9]. Each Petri dish containing 20 mL Sabouraud Agar was kept open for 20 minutes at the mentioned locations, and then closed. Data were recorded for climatic conditions in the last 48 hours, season, time, collection day and type of collection, ambient temperature, relative humidity, type of activities performed by the occupants of the environment, distance between the dish and the ground, and distance between the dish and the ceiling.

Seven distinct areas were evaluated during clinical activities: 1, inside the X-ray room; 2, next to the portable revelation box; 3, in the central corridor that access the dentist room; 4, next to dental cuspidor; 5, next to sink for instrument washing; 6, next to the operator; and 7, next to the handwash sink. The distances of the plates to the ground and to the ceiling were standardized at 80 cm and 190 cm, respectively, being positioned 1 m from the ground. The analyzed clinic has an air conditioner with twenty exits, without circulation or renewal of the air and without HEPA filters.

The plates were then incubated for seven days in a fungi incubator at 26°C and 60% humidity. The fungi growth was monitored daily. The number of colonies was counted on each plate, and data on macroscopic studies (colony size, color, colony appearance) were recorded. Aliquots of the prevalent colonies were sampled in 0.85% sterile saline solution, with dilution of the samples into tubes containing 9.0 mL saline solution to the 10^{-9} dilution in Sabouraud agar plates. The plates were incubated in an oven at 26ºC for five days, to obtain pure cultures. After the incubation period, the colonies were counted and isolated with different characteristics in a test tube containing 9.0 mL slant Sabouraud agar. After incubation, the microculture of the already isolated filamentous fungi was carried out to visualize the reproductive and vegetative structures and subsequent photodocumentation.

After the isolation of the prevalent fungi, samples were sent to the Paraná Institute of Technology (Tecpar) in Curitiba for identification.

3 RESULTS AND DISCUSSION

The environmental conditions and the number of colonies obtained by area are shown in table 1. Areas 6, 2, 3 and 5 presented a higher contamination index, followed by areas 1, 7 and 4. The high index of colonies in these areas is justified by the use of high-speed handpiece, air/water jets and ultrasound, which increase the release of aerosols. Contamination when using these devices is 100% up to one meter away, and 50% at two meters from the patient's mouth[10]. The frequency of cleaning seems to be an important factor in the contamination

of indoor air. It is noted that the reduced frequency of floor cleaning, vacuum pumps and air conditioners increases dirt on these surfaces, which makes the environment contaminated and contaminating. Disinfection, asepsis and sterilization methods are essential in clinics[11].

Among the 411 CFU/mL, the 289 most prevalent were selected and sent for identification. These are illustrated in Figure 1. The description data of the macroscopic identification of the colonies, sampling site and number of colonies per sample are listed in table 2.

Table 1. Climatic conditions and count of colonies by area evaluated

	Day 1	Day 2	Day 3	Total
Season	Winter	Winter	Spring	
Temperature	31° C	26º C	33º C	
Relative humidity	No rain, 20%	Cloudy, 40%	No rain, 20%	
Area 1	39 colonies	7 colonies	2 colonies	48 colonies
Area 2	54 colonies	14 colonies	3 colonies	71 colonies
Area 3	43 colonies	23 colonies	4 colonies	70 colonies
Area 4	25 colonies	12 colonies	2 colonies	39 colonies
Area 5	52 colonies	13 colonies	3 colonies	68 colonies
Area 6	44 colonies	29 colonies	2 colonies	75 colonies
Area 7	24 colonies	11 colonies	5 colonies	40 colonies
Total on the day	281 colonies	109 colonies	21 colonies	411 colonies

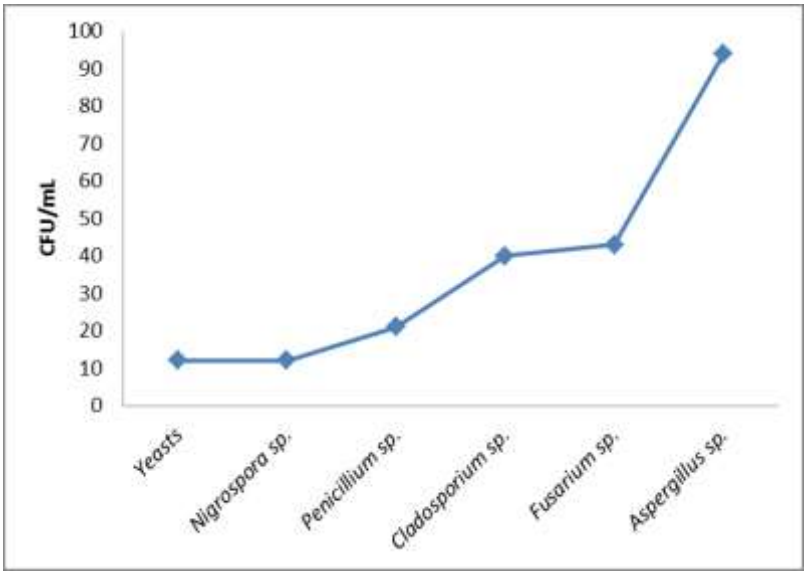

Figure 1. Most prevalent colonies identified in the study

Table 2. Macroscopic identification of colonies, sampling site and number of colonies per sample

Sample	Macroscopic Description	Area	Number	Micromorphological Result
01	Cottony appearance, with light, regular and whitish edges, dark green color	1, 2, 4, 5	18	Filamentous fungus not identified with contaminated material
02	Cottony appearance, with irregular, whitish edges, and pink center	1, 3, 7	12	*Nigrospora* sp
03	Cottony appearance, with light, regular and defined edges, light brown color, a light green core and a white dot at the center	1, 3, 4, 7	13	*Aspergillus* sp
04	Cottony appearance, with light, regular and well-defined edges, dark green color and a deep dark green center	2, 3, 4, 6, 7	28	*Aspergillus* sp
05	Cottony appearance, with light, regular and well-defined edges, dark green color and dark green center	2, 3, 4, 6, 7	30	*Aspergillus* sp
06	Cottony appearance, with light, regular and defined edges, white color and brown center	1, 2, 3, 5, 7	11	*Penicillium* sp
07	Cottony appearance, with light green and white, regular and defined edges, and dark green color	1, 2, 3, 4, 5, 6, 7	23	*Aspergillus* sp
08	Cottony appearance, with light and defined edges, pink salmon color	1, 2, 6, 7	25	*Fusarium* sp
09	Milky appearance, well-defined, pink color	2, 4, 6, 7	12	Microorganism with characteristics of Gram-positive bacilli
10	Cottony appearance, with light, regular, whitish edges, light green color	1, 3, 5, 6, 7	10	*Penicillium* sp
11	Cottony appearance, with light, regular and well-defined edges, green color and with a darker center	1,2, 4, 6, 7	21	*Cladosporium* sp
12	Cottony appearance, with light and defined edges, pink salmon color	1, 2, 6, 7	18	*Fusarium* sp
13	Milky appearance, with defined and opaque edges, white color, with a small beige nucleus	1, 2, 3, 4, 5,6	22	Microorganism with characteristics of bacterium (Gram-positive bacilli)
14	Cottony appearance, with clear, regular and well-defined edges, green color with a darker nucleus	2, 3, 6, 7	19	*Cladosporium* sp
15	Cottony appearance, with regular, white and well-defined edges, light green color	1, 3, 4, 6	15	Filamentous, septate, dematiaceous, non-identified fungus
16	Verrucous appearance, with defined and irregular edges, beige color.	2, 3, 4 ,5	12	Microorganism with yeast characteristics

In samples of air collected outdoors, species belonging to the genera *Aspergillus*, *Penicillium* and *Cladosporium* are common. These genera are also predominant in indoor environments indicating a strong correlation in the presence of fungi between indoor and outdoor air[12].

According to Resolution RE 570 of October 3rd, 2002, of the National Health Surveillance Agency (ANVISA), the presence of pathogenic and toxigenic fungi in indoor environments that are artificially heated, for public and collective use, is unacceptable. Regarding the quantity of non-pathogenic and toxigenic fungi, the permitted limit is 750 CFU/m³ of fungi[7].

In a study previously performed also in a pediatric dentistry clinic, the genera *Aspergillus* and *Penicillium* were found in a greater number of colonies in the indoor environment. Although the most common genera have been saprophytes, including those living on decomposing plant material, *Cladosporium*, *Alternaria*, *Epicoccum* and *Aureobasidium*, the soil-based species such as *Aspergillus* and *Penicillium* have been relatively low in outdoor air but have been found at increased levels indoors[5].

Among the 16 samples sent for identification, 4 belonged to the genus *Aspergillus*. Most of these fungal genera are known to be opportunistic pathogens that can lead to chronic lung diseases or allergic diseases in hosts[3]. In healthy individuals, *Aspergillus* conidia are efficiently eliminated by macrophages and neutrophils. However, when the host is immunocompromised, such as neutropenia induced by chemotherapy or through the chronic use of corticosteroids, the inhalation of *Aspergillus* conidia (spores) can cause a spectrum of diseases such as invasive aspergillosis and chronic necrotizing pulmonary aspergillosis[13]. Thus, in a clinical setting where care is provided to children, air quality control is essential for disease prevention.

Spores of *Aspergillus*, *Penicillium* and *Cladosporium* are prevalent in aquatic and atmospheric environments. Especially some of the *Aspergillus* species like *A. fumigatus* and *A. versicolor* have toxigenic and infectious characteristics[3].

The genera *Aspergillus*, *Penicillium* and *Cladosporium* have potential involvement in Sick Building Syndromes[12]. The presence of the genera *Aspergillus* and *Penicillium* was already expected since they are common in external and internal environments[15].

Clinical manifestations related to *Penicillium* species include superficial and invasive infections as well as allergies. Infections in humans are mainly related to host immunity, and may present more severe alterations in immunocompromised individuals, favoring infectious processes[16].

The genus *Cladosporium* is commonly found in nature, but it is able to grow on surfaces of indoor environments with low levels of humidity, especially those with artificial climatization[15]. *Cladosporium* species are not reported as mycotoxin-producers, but they are a threat to health[12] because they are associated with cutaneous, nail and brain lesions[17]. The genus *Fusarium* is also an agent that causes severe respiratory infections in immunocompromised individuals[18].

In a dental clinic, the main source of contamination is the patient's mouth[10] and the main activity of the dental surgeon is to remove contaminated tissue with body fluids - blood and saliva[11]. Knowing this, measures to avoid cross-contamination in the dental environment are essential. This contamination occurs through air, objects or from person to person, and to prevent it, strict biosecurity precautions are needed[10].

For the solution of unfavorable effects

of fungi in public health, further research on indoor environments is necessary[3]. Seasonal variation may occur in relation to the colonized area with different fungal genera of *Penicillium* and *Cladosporium* from spring to summer, with a decrease in *Penicillium* and increase in *Cladosporium*. Thus, it is expected that fungal genera will present different concentrations in the seasons, with a predominance according to genus and environment, which justifies the different number of colonies reported in the study[19].

Both the temperature and the water availability affect the growth and sporulation of the aerial hyphae, with higher ambient temperature and water available favoring a faster growth. The release of spores is further augmented by intermittent periods of dryness, where the spores are dispersed, and humidity, allowing greater growth and sporulation. The fungal species commonly found reflect the outdoor environment, although concentrations may change seasonally or locally, where the indoor environment favors the growth of specific species[4]. This condition refers to the results reported in this study, in which the internal environment of the pediatric dentistry clinic presents usual conditions for fungal propagation in the environment, since different fungal genera have been identified, with prevalence of the genera *Aspergillus* and *Penicillium*.

The dental environment studied was presented as high risk for fungal contamination. Thus, it is necessary to control the air of the clinic environment continuously, so that the influence of climatic seasons can be monitored, as well as the cleaning and disinfection processes used in the environment.

In order to ensure adequate biosecurity, adequately trained human resources are needed to promote changes in health services, as well as educational and research institutions have to prevent, reduce or eliminate the risks inherent in the health professions[20]. In this way, it will be possible to implement adequate preventive measures, since the presence of pathogenic and toxigenic fungi in the health environment is unacceptable.

The use of HEPA filters may aid in the retention of impurities, reducing contaminants through aerosols present in the environment. Nevertheless, their use is not yet routine. This study may help to instruct professionals about their use. As well as guidance should be given to cleaning professionals regarding the frequency in the cleaning and maintenance of the equipment/device and to dental professionals with respect to the correct use of personal protective equipment.

When performing small or large surgeries, the dental surgeon should be aware of contamination coming from the air, as this is also important for the postoperative period to be successful and without complications for the patient. By knowing the fungal genera to which their patients are exposed, the professional can minimize the risks of infection by air, adopting practices of cleaning the environment and air that can help in the prevention of postoperative fungal infections.

4 CONCLUSION

In this study, it was shown that the most prevalent fungal genus was *Aspergillus*, which could represent a significant risk for lung problems and trigger allergic processes in the most vulnerable populations. The other genera found in descending order were *Fusarium*, *Cladosporium*, *Penicillium* and *Nigrospora*, all recognized as agents of possible opportunistic infections for patients with reduced immunity. The present study

also contributes to the evaluation of air quality; however, it is evident the need for randomized controlled trials to aid in the decision making for clinical practice.

RESUMO

Identificação da população fúngica em amostras de ar coletadas em clínica de ensino de Odontopediatria

Agentes infecciosos podem ser transmitidos via partículas microscópicas suspensas no ar e secreções presentes em superfícies contaminadas de instrumentos e equipamentos. Entre os contaminantes veiculados pelo ar em ambiente interno estão os fungos, bactérias e vírus, que são provenientes do ar externo, do sistema de climatização, da construção, mobiliário, carpete e de seus ocupantes. A maioria dos procedimentos realizados na clínica odontológica libera grande quantidade de aerossóis, partículas diminutas que ficam durante várias horas no ar e podem conter diversos micro-organismos, dentre eles, os fungos. Em clínicas de ensino, esta contaminação é maior devido ao alto número de ocupantes e de procedimentos realizados ao mesmo tempo. Ao conhecer os gêneros fúngicos aos quais seus pacientes estão expostos, o profissional pode minimizar os riscos de infecção, adotando práticas de higienização do ambiente e do ar que possam auxiliar na prevenção de doenças. A verificação de fungos foi realizada pelo método de sedimentação em placa, que se mostra útil para a análise da quantidade e da qualidade de fungos presentes em ambientes internos e externos. Foram identificadas espécies pertencentes aos gêneros *Aspergillus, Fusarium, Cladosporium*, *Nigrospora* e *Penicillium*, encontrados com maior frequência os gêneros *Penicillium* e *Aspergillus* provenientes de ambiente externo e interno.

Descritores: Infecções Bacterianas e Micoses. Aerossóis. Clínicas Odontológicas. Odontopediatria. Controle de infecções.

REFERENCES

1. Zemouri C, de Soet H, Crielaard W, Laheij A. A scoping review on bio-aerosols in healthcare and the dental environment. Plos One. 2017;12(5):e0178007. DOI: 10.1371/journal.pone.0178007.
2. Brasil. Lei de Diretrizes e Bases da Educação Nacional. Lei nº 9.394/1996 – Lei nº 4.024/1961 artigo 43. Senado Federal. Brasília: Coordenação de Edições Técnicas; 2017.
3. Kadaifciler D, Cotuk A. Microbial contamination of dental unit waterlines and effect on quality of indoor air. Environ Monit Assess. 2014; 186(6):3431-44.
4. Boch T, Reinwald M, Postina P, Cornely O, Vehreschild J, Spiess B, et al. Identification of invasive fungal diseases in immunocompromised patients by combining an Aspergillus specific PCR with a multifungal DNA-microarray from primary clinical samples. Mycoses. 2015; 58(12):735-45.
5. Rogawansamy S, Gaskin S, Taylor M, Pisaniello D. An evaluation of antifungal agents for the treatment of fungal contamination in indoor air environments. Int J Environ Res Public Health. 2015; 12(6):6319-32.
6. Brasil. Cartilha de proteção respiratória contra agentes biológicos para profissionais da saúde. Brasília: Ministério da Saúde, 2009.
7. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução nº 9, de 16 de janeiro de 2003. Orientação técnica sobre padrões referenciais de qualidade do ar interior em ambientes climatizados artificialmente de uso público e coletivo. Diário Oficial da União, Brasília, DF, 20 jan. 2003.
8. Barreto ACB, Vasconcelos CPP, Girao CMS, Rocha MMNP, Mota OML, Pereira SLS. Contaminação do ambiente odontológico por aerossóis durante atendimento clínico com uso de ultrassom. Braz J Periodontol. 2011; 21: 79-84.
9. Jurado S, Bankoff A, Sanchez A. Indoor air

quality in Brazilian universities. Int J Environ Res Public Health. 2014; 11(7): 7081-93.
10. Sousa KS, Fortuna JL. Microrganismos em ambientes climatizados de consultórios odontológicos em uma cidade do extremo sul da Bahia. Rev Baiana Saúde Pública. 2011; 35(2): 250-63.
11. Segers F, Meijer M, Houbraken J, Samson R, Wösten H, Dijksterhuis J. Xerotolerant Cladosporium sphaerospermum are predominant on indoor surfaces compared to other Cladosporium Species. Plos One. 2015; 10(12):e0145415. DOI: 10.1371/journal.pone.0145415.
12. Kolwijck E, van de Veerdonk F. The potential impact of the pulmonary microbiome on immunopathogenesis of Aspergillus-related lung disease. Eur J Immunol. 2014; 44(11): 3156-65.
13. Hayleeyesus SF, Manaye AM. Microbiological quality of indoor air in university libraries. Asian Pac J Trop Biomed. 2014; 4(1):321-17.
14. de Sant'anna JB, Santos CF, Carneiro JA, Coutinho FN. Investigação de fungos anemófilos em ambiente cirúrgico do Hospital Regional de Brazlândia-DF. Proceedings COPEC. 2010; 7(10): 409-12.
15. Guevara-Suarez M, Sutton D, Cano-Lira J, García D, Martin-Vicente A, Gené J, et al. Identification and antifungal susceptibility of Penicillium-like fungi from clinical samples in the United States. J Clin Microbol. 2016; 54(8): 2155-61.
16. Melo LLS, Lima AMC, Damasceno CAV, Vieira ALP. Flora fúngica no ambiente da Unidade de Terapia Intensiva Pediátrica e Neonatal em hospital terciário. Rev Paul Pediatr. 2009; 27(3):303-8.
17. Haleem Khan AA, Mohan Karuppayil S. Fungal pollution of indoor environments and its management. Saudi J Biol Sci. 2012; 19(4):405-26.
18. Frankel M, Hansen E, Madsen A. Effect of relative humidity on the aerosolization and total inflammatory potential of fungal particles from dust-inoculated gypsum boards. Indoor Air. 2014;24(1):16-28.
19. Neder RN. Microbiologia: manual de laboratório. São Paulo, Brasil: Editora Nobel; 1992.
20. Ministério da Saúde (BR). Biossegurança em saúde: prioridades e estratégias de ação. Brasília: Ministério da Saúde; 2010.

Correspondence to:
Lígia Maria Molinari Capel
e-mail: ligia.capel@unicesumar.edu.br
UniCesumar
Av. Guedner, 1610 Jardim Aclimação
87050-390 Maringá/PR Brazil